Approaches for delaying sexual maturation in salmon and their possible ecological and ethical implications

Short title: Implications of delaying sexual maturation in salmon

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Abstract
The aquaculture industry is under pressure to satisfy global demands for marine foods. Atlantic salmon has been bred for more than forty years, and substantial progress has been made within the culturing and breeding programs. The improved growth rate of Atlantic salmon has been accompanied by an earlier onset of maturation. Among the factors controlling maturation in salmon is photoperiod, temperature and body composition. Early sexual maturation is detrimental to fish health and quality when viewed from an aquacultural viewpoint. There are several approaches for alleviating this problem 1) traditional breeding selection, 2) manipulating external factors affecting puberty (f. ex. light), 3) novel biotechnological methods improving breeding methods, 4) induction of polyploidy, and 5) genetic modification controlling maturation. We present ecological and ethical issues connected to these approaches, and argue the importance of acknowledging and discussing such issues in order to ensure that all stakeholder concerns are considered.

Keywords: aquaculture, biotechnology, ecosystem, ethics, animal welfare, consumer interest

1 Introduction

Aquaculture has developed rapidly over the last decades in order to meet the growing demand for marine foods, a demand that cannot be met by increased harvest of wild stocks as they already are under stress from commercial fisheries (Gjedrem et al., 2012, Gillund et al., 2008, Bostock, 2011). Many species, ranging from sea urchins to salmon, are now farmed effectively at a large scale. However, there are at present very few breeding programs established for cultured marine species (Gjedrem, 2012, Gjedrem et al., 2012). The anadromous (shifts between fresh and salt water) Atlantic salmon (*Salmo salar*) is one of the species where breeding programs have been successfully established (Gjedrem, 2012). In Norway salmon has been actively bred for more than four decades (Gjedrem, 2012), and substantial advances have been made with regard to growth-related traits and disease resistance (Gjedrem et al., 2012, Gjedrem, 2012, Thodesen and Gjedrem, 2006). Early sexual maturation has undesired impacts on health, growth and product quality of farmed fish (Aksnes et al., 1986, Taranger et al., 2010, Fjelldal et al., 2012). In order to avoid these negative effects maturation is commonly delayed by exposing fish to continuous light affecting the perception of season and its circannual rhythm (Taranger et al., 2010). As global warming proceeds, ocean temperatures will increase, particularly in the Arctic region (Serreze and Barry, 2011). Because temperature is also an important *zeitgeber* for initiation of maturation (Bromage et al., 2001, Pankhurst and King, 2010), the increasing ocean temperatures might to some extent overrun the inhibitory effect of light on maturation.

Modern biotechnology provides several possibilities for attempting to delay or prevent maturation in salmon in order to improve production efficiency. These possibilities may affect both the environment and the welfare of salmon and thus poses ecological and ethical challenges. Additionally there are fundamental ethical issues pertaining to the application of modern biotechnology on fish (Ormandy et al., 2011), that need to be discussed on a case-by-case basis, such as the use of modern biotechnology to delay maturation in salmon.

When faced with new opportunities and choices it is important to consider the implications and consequences, as well as their reversibility and irreversibility. What would be the repercussions of choosing one opportunity instead of another? Are the choices mutually exclusive, or could the different directions be combined? The many different stakeholder groups involved in aquaculture (industry, consumers, researchers, government, environmentalists, fishers, etc.) are likely to have contradicting views on the different approaches, and may prioritize differently between for example economical and ethical considerations. When evaluating new biotechnology approaches it is important to keep in mind how the different stakeholder groups interact, and that the ecological and ethical issues by the different approaches for delaying maturation are identified and discussed in open and participative ways.

2 Sexual maturation in salmon –a challenge for aquaculture

Sexual maturation, or puberty, is the process in which a juvenile individual develops into a sexually functional adult with the ability to produce gametes and gains the somatic and behavioral competence to function as a mating partner and a parent (depending on species). Gonadal development in salmon is initiated already before hatching with the initiation of germ cell formation and gonadal development, and continues in stages until final sexual maturation (puberty) occurs. Maturation in salmon seems to be controlled by several intrinsic factors such as size, growth rate and fat deposition, in addition to several extrinsic factors such as photoperiod, temperature, diet, stress exposure and competition (Wild et al., 1994, Thorpe et al., 1990, Taranger et al., 2003, Taranger et al., 2010, Pankhurst and Munday, 2011, Pankhurst and King, 2010, Jonsson et al., 2012, Thorpe et al., 1998). When these intrinsic and extrinsic factors together meet certain conditions, the brain-pituitary-gonad (BPG) axis is triggered and maturation is continued through to the next step. A multitude of genes and their products can be expected to be involved in the process of initiating and continuing the maturation process from one stage to the next. Wild salmon show large genotypic variation between populations and even subpopulations within the same river catchment (Skaala et al., 2004), additionally phenotypic variation can be large between generations due to juveniles experiencing different environmental conditions from year to year. Additionally male salmon exhibit two adult morphs with different body sizes and mating strategies ("sneaking", "fighting") (Fleming, 1996). Farmed salmon often show a phenotypic response to their improved growth conditions where both age and size at puberty is reduced, accompanying the high growth rate and large size achieved through selective breeding (Porter et al., 1999). For this reason it is challenging for breeding programs to select for late maturity as the phenotypic response can mask the genetic variability in age and size at maturity (Taranger et al., 2010).

Sexual maturation, with heavy investments in gonadal development and gamete production, has several negative consequences for growth rate, welfare and product quality of domesticated Atlantic salmon (Stien et al., 2013). Maturation represents a welfare issue in fish farming since the domesticated salmon gradually loses tolerance to seawater due to compromised hypo-osmoregulation ability during maturation (shift from seawater adaptations to freshwater adaptations; hyper-osmoregulation) (Persson et al., 1998, Stien et al., 2013), this may cause dehydration, stress and possible negative effects on the immune system (Harris and Bird, 2000). When the wild salmon begins to mature, they immigrate to their native rivers, an escape that is not possible for domesticated salmon due to their confinement in cages in seawater facilities.

At the onset of puberty growth rate is high (Gjerde et al., 1994), which can be beneficial for the farming industry as somatic growth can be maximized. However, a few months into the maturation process feeding will cease, and somatic weight will decrease as resources are routed into gonadal development and gamete production (Taranger et al., 2010). Aksnes et al. (1986) found that maturation reduced fat and protein content of salmon filets from 12% to 5% and from 22% to 19% respectively. In the same study water content increased during maturation from 66% to 74% (Aksnes et al., 1986). Additional chemical changes cause deterioration in salmon product quality where smell and taste change dramatically (Taranger et al., 2010). Maturation may lead to a loss of flesh pigmentation as the pigment is shifted to skin and gametes (Aksnes et al., 1986, Taranger et al., 2010), reducing its attractiveness to consumers (Alfnes et al., 2006).

The behavior of salmon also changes during sexual maturation. This especially affect males that take on a dominant role during mate competition and become more aggressive toward other males. However, behavioral changes are also observed in females and subordinate males (Taranger et al., 2010). In order to avoid these physical and behavioral changes, farmed fish should be culled before sexual maturation, but industrial and economic demands to slaughter size (4-5 kg) and the individual rate at which a salmon matures makes this difficult.

Finally, when immature salmon escape they are likely to run out to sea where survival is low, subsequently they are less likely to survive to mate with wild salmon. A mature salmon is likely to seek up-river in order to mate (Taranger et al., 2010, Hansen, 2006). Thus keeping the salmon in an immature stage throughout production will not only be beneficial to the industry with regards to product quality and animal welfare, but is also an effort that could benefit the wild salmon strains whose gene pools are less likely to be negatively impacted by domesticated salmon with delayed or inhibited maturation (Taranger et al., 2010, Naylor et al., 2005, Jonsson and Jonsson, 2011, Skaala et al., 2012).

3 Approaches for regulating maturation

There are many ways of approaching the study and regulation of maturation in Atlantic salmon, spanning from traditional to cutting-edge science.

Substantial progress has been made over the last decades in the fields of fish physiology, behaviour, genetics and epigenetics. This knowledge has served current breeding programs in the aquaculture industry in ways to delay or inhibit maturation in domestic salmon. The choice of approach depends on factors such as available methods, costs, consumer acceptance and fish welfare. Both potential methods and factors required to evaluate these methods are presented and discussed in the following sections. For a short summary of the proposed biotechnological approaches to delay or inhibit maturation in domestic Atlantic salmon see table 1.

3.1 Traditional selection and salmon breeding: What traits are selected for and how?

Selective breeding takes advantage of the large genetic variation found for many traits in a population. However, in traditional breeding selection is primarily done on external and easily observable traits (Gjerdrem and Baranski, 2009). Breeding programs involve selection either on just one trait or on several traits. Overall breeding goals for the aquaculture industry can be summarized as a selection for traits that reduce the cost of production, produce a high quality product, satisfies consumer preferences, improve fish welfare, reduce stress and increases disease resistance (Davis and Hetzel, 2000, Gjedrem and Baranski, 2009).

Selective breeding programs for Atlantic salmon have since the early 1970s been effective in achieving their goals of increased growth rate, more efficient feed utilization and disease resistance (Gjedrem, 2012, Gjedrem et al., 2012). Salmon is also bred for improved taste, reduced aggressiveness and tameness. These traditional selective breeding programs have been very successful, but have been extremely time-consuming.

Although traditional selection is mainly possible for observable traits, some selected traits might require testing causing disqualification of the individual from breeding (for example exposure to disease). In the latter case siblings of the individuals tested are still eligible for breeding, but morphologically there is no way to investigate whether they have the same combination of genes (Gjedrem and Baranski, 2009). Aquatic species show a much higher level of responsiveness to selection than terrestrial livestock, even if general heritability is the same. This is due to the higher fecundity of fish and the larger variation within the preferred traits (Gjedrem and Baranski, 2009, Fjalestad et al., 2003).

Genetic correlation between traits generally contributes to genetic stability when related traits are co-selected. Occasionally the selected trait will be correlated with a trait that is outside of the breeding goal, potentially having a negative impact on fish quality, welfare and health in the context of aquaculture (Gjedrem and Baranski, 2009). Sexual maturation is strongly connected to growth rate, and selection for faster growth leads to more incidences of early maturation (Gjerde et al., 1994, Taranger et al., 2010). However, Gjerde and Korsvoll (1999) reported a 3% genetic gain per generation when selection against early maturation, while still maintaining a 13-15% genetic gain per generation with selecting for growth. Thorpe et al. (1983) conclude in their study of inheritance of developmental rates in salmon that the best method for achieving rapid growth and late maturation is by selecting for rapid development while manipulating the environment in order to suppress maturation (photoperiod, temperature). With increasing sea temperatures, it is expected that it will become increasingly difficult to delay maturation by photo manipulation.

3.2 Environmental factors and external cues and their effect on maturation

Environmental factors and external cues, such as food access, temperature and photo manipulation are approaches that are established to varying degrees for delaying maturation. These methods are cost-effective but can be time consuming due to monitoring requirements and administration.

The timing of maturation events in Atlantic salmon has been linked to physical condition and could possibly be connected to certain set-points or ratios of lipid and protein mass (Thorpe et al., 1990, Thorpe et al., 1998) interacting with external cues, and in particular photoperiod, for initiation of sexual maturation (Melo et al., 2014, Andersson et al., 2013, Benedet et al., 2010, Vikingstad et al., 2008). The high plasticity of salmon seems to allow for specific time windows (signaled through photoperiod) during which the salmon can initiate the sexual maturation process or wait depending on body condition and ambient circumstances (Thorpe et al., 1990, Thorpe et al., 1998).

3.2.1. Effect of feed manipulation and vaccination on maturation

Diet restrictions around the time when maturation is initiated, or not, has proven to prevent maturation, however, it also reduces growth rate and is not a very desirable method in terms of animal welfare (Vainikka et al., 2012, Jonsson et al., 2012). Factors such as reduction in lipids or increased phosphorus level in the diet might also influence the timing of maturation (Fjelldal et al., 2012, Jonsson et al., 2012). Changing the diet of farmed salmon can have implications for welfare, and the various diets should be carefully evaluated in order to avoid health issues and a degradation of product quality.

Jonsson et al. (2012) found that Atlantic salmon reared in heated water and fed a high lipid diet could grow less during winter and still attain maturity, compared to conspecifics reared in cold water. In a different study it was found that when female salmon was fed a low lipid diet fewer matured in the warm water than in the cold water set up (Vainikka et al., 2012).

A study by Thorpe (1990) found that feed restriction over the winter month reduced the proportion of maturing fish in the studied cohorts. All studies point to there being a complex set of interactions between environmental conditions, food access and body condition that leads to the initiation of puberty in Atlantic salmon.

Vaccinations have been connected to short-term loss of appetite and reduced growth (Sørum and Damsgård, 2004), hence it is also probable that being vaccinated (any vaccine) has an effect on metabolism and can be used to reduce incidences of early maturation (Fjelldal et al., 2012).

3.2.2. Effect of photoperiod manipulation on maturation

Many studies have shown the effect of exposure to artificial light/dark cycles on the onset of maturation; hereby opening an opportunity to postpone maturation by keeping the fish under a light regime not providing photoperiodic cues that could induce maturation (Adams and Thorpe, 1989, Taranger et al., 1998, Porter et al., 1999, Melo et al., 2014). In a study by Porter et al. (1999) only 6.1% matured under an artificial light regime (exposed to additional light during night) compared to 61.5% maturing in the control group exposed to the natural light regime. The extended cue of changing day length is missing, preventing the onset of maturation. Porter et al. (1999) found that melatonin levels is linked to maturation, and has proposed that the initiation of maturation depends on a threshold level of plasma melatonin being reached. The aquaculture industry has successfully manipulated both growth and initiation (as well as progress of) maturation in salmonids by controlling the light:dark cycle of young fish (Endal et al., 2000, Arge et al., 2014, Taranger et al., 2010, Oppedal et al., 2011).

3.3 Regulatory pathways controlling maturation in salmonids

An understanding of the regulatory pathways controlling maturation events in salmon provides a good baseline for further experimental investigation. Detailed knowledge on the maturation process is continuously gained by researchers, and could in combination with technological advances (Edvardsen et al., 2014, Wargelius et al., 2016) lead to efficient ways for postponing or even blocking maturation in salmon.

For example, knowledge about signals and mechanisms initiating and driving the maturation process (e.g. activation of the brain-pituitary-gonad axis, activation of steroidogenesis in gonads, and effects of growth and adiposity factors) can be used to develop ways to control the maturation process in such a way that fish health and quality is not deteriorated (Andersson et al., 2013, Benedet et al., 2010, Melo et al., 2014, Taranger et al., 2010).

Research efforts that measure and track hormonal levels (for example of gonadotropin-producing hormone, melatonin, growth hormone and luteinizing hormone) in salmon over time and under varying conditions can provide good information on how the external environment affects the expression of hormones and other signaling molecules in salmon (Taranger et al., 2003, Bromage et al., 2001, Trombley and Schmitz, 2013).

3.4 Biotechnology –new tools and new methods for investigation and manipulation

3.4.1. Induced polyploidy

The term polyploidy refers to a condition where an individual has more than two sets of chromosomes. Polyploidy is usually lethal for mammals and birds, but commonly observed in plants, fish and amphibians. Triploidy (three sets of chromosomes) is relatively easy to induce by shocking the fish embryos with hydrostatic pressure or exposing them to drastic temperature changes. This causes the second polar body to be retained within the fertilized egg (the second polar body is a term used to describe a set of maternal chromosomes that normally leave the fertilized egg at the completion of meiosis). Tetraploid fish with two extra chromosome sets can be produced using similar inexpensive methods, and when bred with diploids they sire 100% triploids for farming (Benfey, 2001). Triploid and tetraploid fish have bigger, but fewer cells, due to the additional space required by the additional chromosomes (Small and Benfey, 1987).

Triploid fish (and shellfish) have been introduced in several countries across the world for aquaculture purposes, and are available for consumption (Rasmussen and Morrissey, 2007, Piferrer et al., 2009). Most of these fish appear healthy under optimal growth conditions (Benfey, 2015, Fraser et al., 2013, Fraser et al., 2012a). Triploidy is generally seen as a way of improving growth, health and quality of farmed fish, as energy will be invested in these three factors only, and not in maturation. However, some species and strains of fish do experience adverse health issues, as morphological, physiological and behavioral irregularities have for example been reported for salmon (Fraser et al., 2012a, Dunham, 2004, Fraser et al., 2013, Benfey, 2001, Fraser et al., 2015a, Fraser et al., 2015b, Taylor et al., 2013, Fraser et al., 2012b). The observed health problems in triploid salmon can be due to suboptimal rearing conditions (Fraser et al., 2012a), hence animal welfare can be improved by optimizing diet and conditions.

Induction of triploidy is likely to be 100% effective over time, and triploid salmon that escape will be unable to reproduce with the wild strains. However, male triploid fish will still show reproductive behavior on the mating grounds (but produce non-viable sperm cells), and interact with the wild salmon (Fjelldal et al., 2014, Fraser et al., 2012a, Piferrer et al., 2009), i.e. competing for mating's (i.e. roe might be left unfertilized) and resources such as habitat and prey.

So far Atlantic salmon triploids have not shown superior performance when compared to diploids, but they have shown survival and growth that is similar to or better than diploids under experimental settings (Oppedal et al., 2003). Benfey (2001) suggests that polyploidy varieties of cultured species should be considered "new species" within aquaculture because they might require other production regimes (i.e. they have different environmental requirements), and receive support from Fraser et al. (2012a) in that more efforts should be made to specify their optimal rearing conditions. Whether or not the products from triploid fish should be distinguished, for example through labeling from that of diploid fish, in order to safeguard consumer rights is still an open question that needs to be discussed.

3.4.2. -Omics as a tool

During the last decade further insight into genetics and epigenetics, together with innovations in proteomics, metabolomics and transcriptomics, have been gained and have greatly improved the understanding of fish genetics, fish physiology, the mechanisms affecting quality traits (pigmentation, fat deposition), disease resistance, and the effect of nutrition on general fish health (Lokman and Symonds, 2014, Rodrigues et al., 2012). Current developments include better understanding of skeletal malformations, infectious disease, stress, toxicology, antibiotics, probiotics and vaccines in farmed aquatic species.

Proteomics, together with transcriptomics and metabolomics, are robust tools screening for proteins, RNA and genes, and for investigating the pathways and functional networks that control growth and maturation in fish. Growth and maturation are processes governed intrinsically by genes, body composition and metabolic state, and by environmental factors. Although expensive to establish and perform, the –omics technologies provide important insight into the effects of exogenous and endogenous variables on regulation of growth.

3.4.3. Marker assisted selection

DNA markers and genetic modification opens up vast possibilities for genetic changes in aquaculture species (Edvardsen et al., 2014, Fjalestad et al., 2003, Hayes and Andersen, 2005). Marker assisted selection (MAS) is a genotype based method for selecting brood stock. Instead of targeting the phenotype, selection is performed on genotype or on a combination of value estimates based on marker genotype and phenotypic trait data. This requires a good understanding of the genes affecting a trait, the interaction and linkage between these genes and with genes controlling other traits. For example, genetic studies mapping genes involved in resistance to infective pancreatic necrosis (IPN) have been successful, and their results have been used in MAS aiming at building resistance against this disease in Atlantic salmon (Moen et al., 2009, Houston et al., 2008, Robertsen, 2011, Gheyas et al., 2010). Once the role of genes involved in the regulation of sexual maturation has been elucidated, MAS can be a useful tool. Recent studies of quantitative trait locus (QTL) and single-nucleotide polymorphisms (SNP's) in Atlantic salmon have revealed genetic regions and QTLs containing genes suspected to be involved in the regulation of sea age (indicating the number winters spent in the sea) and migration, and age at sexual maturation (Johnston et al., 2014, Gutierrez et al., 2014). With time, these findings will prove useful genetic markers in selection for higher age at sexual maturation.

MAS is a work intensive method, though likely to provide good long-term results when breeding for complex traits. New technology such as SNP chips is expected to reduce both the labor requirements and costs of MAS. The full genome of the Atlantic salmon has recently been mapped by the International Cooperation to Sequence the Atlantic Salmon Genome (ICSASG). Once the genes involved in regulation of sexual maturation have been revealed, further opportunities for using MAS will be available.

3.4.4. Genetically modified salmon

Improvements of genetic engineering techniques and functional genomics lead to the development of genetically modified (GM) fish. This encompasses modifying growth rate, feed utilization, disease resistance, stress tolerance, and maturation processes, thereby enhancing the quality and cost effectiveness of the farmed salmon. It can also lead to developing new products through alterations of the salmon genome (Edvardsen et al., 2014, Melamed et al., 2002).

In particular, the ability to increase growth rate by introducing growth hormone (GH) genes has been applied frequently in genetic modification of fish (FAO, 2003). Research by AquaBounty Technologies has resulted in the *AquAdvantage*, a GM Atlantic salmon with a inserted gene construct composed of the regulatory elements of an antifreeze protein gene from ocean pout (*Zoarces americanus*) controlling the expression of a GH gene from chinook salmon (*Oncorhynchus tshawytscha*). This makes the *AquAdvantage* salmon grow non-stop, thus reaching a suitable culling size faster than wild-type salmon. The *AquAdvantage* salmon was recently approved (November, 2015) by the US Food and Drug Administration (FDA) under the New Animal Drug Provision of the Federal Food, Drug and Cosmetic Act, and the National Environmental Policy Act. The FDA and the Veterinary Medicine Advisory Committee had already concluded in 2010 that the *AquAdvantage* salmon "is as safe as food from conventional Atlantic salmon" and that it is "not expected to have significant impact on the quality of the human environment". However, feedback to the FDA from NGOs and other independent institutes led to a delay in the processing of the application as concerns raised by these groups had to be addressed before a final approval could be made (Van Eenennaam and Muir, 2011, Fox, 2010). Protests were issued to that the *AquAdvantage* salmon had been evaluated under the FDA's animal drug approval process due to the lack of a separate system for evaluating GM animals. Concerns about allergenicity and hormone content of the GM salmon have also been raised, as well as issues related to risk towards wild salmon stocks and the environment. Under the considered evaluation grid, the *AquAdvantage* salmon did not require to be labeled as GM (because the FDA did not find it to be substantially different from non-GM salmon) however, only a month after approval, the American congress passed a bill directing the FDA to develop a separate labeling system for GM salmon. The approval has hence been withdrawn and held up awaiting a functional labeling system.

Devlin et al. (2001) found that rainbow trout genetically modified to overexpress growth hormone did not grow any faster or larger than a commercial strain of rainbow trout which had undergone traditional selection for fast growth. Also, it was found that some of the GM fish had deformities and reduced viability whether the transformed strain came from a wild or a domestic background. This shows that growth is a complex feature controlled by many genes and external factors. This gives reason to thoroughly evaluate each case of genetic modification, and decide whether it is the most appropriate approach to achieving the breeding goals.

The possibility to alter or knockout the function of genes in salmon has been enabled through the establishment of targeted mutagenesis technology (such as zinc-finger, TALENs and CRISPR/Cas technologies) in fish (Edvardsen et al., 2014, Meng et al., 2008, Doyon et al., 2008, Huang et al., 2011, Hwang et al., 2013). This technology allows for small changes in the salmon genome and will be a valuable research tool for investigating important production traits, such as precocious maturation, disease resistance, and immune parameters, among others. Eventually, targeted mutagenesis technology can also be of interest for breeders wanting to delay maturation. Recently researchers have produced a salmon where an essential protein for gamete production has successfully been knocked out using CRISPR/Cas technology (Wargelius et al., 2016). The identification of this essential protein and the gene that controls it provides the possibility to produce a DNA vaccine to delay the sexual maturation.

There are several issues connected to the application of genetic modification and the new possibilities arising from further development of biotechnology (such as CRISPR/Cas). As with other GMOs, the production and use of GM animals require time-consuming risk assessments. How to evaluate and regulate the safety and welfare of animals and other organisms developed or modified through the use of modern biotechnology, such as GM and CRISPR/Cas still remains an uncertain issue. The production of GM salmon raises similar ethical issues as the production of GM crops, namely those related to reduced autonomy of impacted parties (i.e. the species itself, producers, and consumers), ownership, consumer concerns and unwanted environmental impact (Le Curieux-Belfond et al., 2009, Hursti et al., 2002, Frewer et al., 2011).

3.4.5. DNA vaccination

DNA vaccines are purified bacterial plasmid preparations containing one DNA sequence or more, that express a protein antigen (antigens) or peptides capable of inducing an immune response (Pereiro et al., 2014). The process required for the development of DNA vaccines is relatively simple, which entails that when the process is established, it can be generic for various purposes.

Traditionally, DNA vaccines have been intended to hinder infectious diseases by producing antigens that boost the immunological response. Recently, DNA vaccines have also been targeted against non-infectious health issues such as cancer and rheumatisms (Juan et al., 2015, Pol et al., 2014). In principle, the DNA vaccine could also be targeted against specific physiological processes involved in sexual maturation, hindering or postponing the onset of maturation in domestic salmon.

The main safety aspects of DNA vaccines are unwanted effects (off-target effects) on the vaccinated fish. These effects include development of autoimmunity, host cell genome integration, inflammation of injection site, resistance to antibiotic resistance genes, and tissue destruction (Gillund et al., 2008, Pereiro et al., 2014). Environmental safety aspects include potential shed of the vaccine from the vaccinated fish to the environment or by predatory animals eating the vaccinated fish (Gillund et al., 2008). Due to the many safety aspects, the regulatory path towards approval of DNA vaccines is long and time-consuming. The development of vaccines moreover requires the culling of many fish.

3.4.6. Overview of biotechnology approaches to delaying sexual maturation in salmon

MAS and genetic engineering strategies seem to be the most efficient techniques to engineer the salmonid genome. Though similar in their specificity when it comes to the selection of desirable genes, the gene pool from which to select genes differs substantially. While MAS is limited to utilizing the existing genetic variation intrinsic of the species, genetic modification can attempt to utilize genes from every known organism. However, genetic modification is currently limited to traits that can be changed through the presence, change or absence of simple gene constructs, while MAS interferes in the genome by using only the genes intrinsic to the species.

4 Ecological issues

All of the available methods for delaying maturation raise ecological issues. For an overview of benefits, ecological issues, and ethical aspects linked to the different approaches described for delaying maturation in salmon see table 2.

4.1 Impacts on the environment surrounding the aquaculture facilities

Aquaculture will always influence the surrounding environment including the local coastal areas. The ecological impact will depend on the cultured organism and method of aquaculture utilized, in addition to local conditions (climate, currents etc.). Some forms of aquaculture might also have regional or global impact, for instance if feed comes from intensive offshore fisheries. Innovations in salmon farming need to be assessed and monitored for their environmental impact. Feed additives given in order to affect the maturation process may come into contact with other free-swimming and sessile organisms in and around the production facility (e.g. detritivore organism feeding on aquaculture waste), and hence have an impact on the ecosystem surrounding the aquaculture production facility. Feed additives such as phosphorus (Fjelldal et al., 2012) could also indirectly lead to changes in water quality (eutrophication) due to its importance in the marine system. Hormonal additives in the feed could have disturbing endocrine effects on various detritivores and opportunistic marine creatures in and around the aquaculture facility (Depledge and Billinghurst, 1999, Rodríguez et al., 2007, Oberdörster and Cheek, 2001). In general additives, including chemical, hormonal and medicinal (such as antibiotics and probiotics) should be evaluated on a case-to-case basis taking into consideration the nature of the local ecosystem and potential for non-target effects, such as bioaccumulation and transportation of the additive.

4.2 Environmental impacts of escapes

An important adverse environmental impact of sea-based aquaculture is the escape of domesticated strains of cultured species. The Norwegian Institute for Marine Research (IMR) considers, in their 2013 review of Norwegian aquaculture, that the reported amount of escaped fish is a minimum estimate (Taranger et al., 2014). According the IMR's own estimates, 1-1.5 million salmon escape from Norwegian aquaculture facilities every year. This estimate is 4-5 times as high as reported, the large discrepancy is thought to be due to undetected and unreported escapes of smolt (Taranger et al., 2014). In both the Atlantic and Pacific oceans the escaped farmed salmon compete with the wild salmon for food, habitats and spawning grounds, and they could also be spreading parasites as sea louse and diseases to other aquaculture facilities as well as the wild salmon (Skaala et al., 2012, Jonsson and Jonsson, 2011, Fisher et al., 2014, Torrissen et al., 2013).

Immature escaped salmon will normally swim out to the open sea, and only seek back to the coast and upriver once it sexually matures. How quickly it leaves the fjord system is, however, seasonally dependent, and may also be influenced by the production regime the escaped salmon was under (Taranger et al., 2014, Skilbrei et al., 2014, Skilbrei et al., 2015). Both domesticated and wild salmon have poor survival at sea (Taranger et al., 2014). The younger the salmon is when it escapes the more similar to wild salmon it becomes as it grows, i.e. it adapts much better to life in the wild (Taranger et al., 2014). Both the mating success and offspring survival of domesticated salmon in the wild is low compared to that of the wild salmon (Fleming, 1996, Garant et al., 2003, Weir et al., 2004, Skaala et al., 2012). Because of their high growth rate, some studies suggest that offspring of domesticated salmon might be at a size-related advantage through certain life stages in the wild (McGinnity et al., 2003, Fleming et al., 2000).

Escaped domesticated fish strains could influence the ecosystem and wild conspecifics in multiple ways (Taranger et al., 2015). The environmental impact depends on the amount of escaped domestic salmon, their phenotypic characteristics related to survival over time, the ability for reproduction, and on how they adapt to the aquatic biodiversity present in the receiving ecosystem (Kapuscinski and Hallerman, 1991). Ability for survival and reproduction in fish are dependent on six traits; juvenile viability (chances of surviving to sexual maturity), adult viability (chances of surviving to procreate), fecundity, fertility (percent of eggs successfully fertilized by male sperm), mating success and age at sexual maturity (Muir and Howard, 1999, Muir and Howard, 2002, Muir and Howard, 2001).

Concerning genetic interactions between escaped domesticated and wild salmon, Verspoor et al. (2006) concluded that the impact can be both direct (physiological and metabolic changes due to crossbreeding) or indirect (competition, disease and parasite interactions). The size of the impact will depend on the actual inflow of farmed fish, resulting gene flow and the condition of the wild population. Verspoor et al. (2006) suggest that well-planned site selection of fish farms, with adequate distances from wild salmon habitats and effective containment of the farmed salmon, can minimize incidents and impact of escapees, including the risk of spreading disease. Another option is to maximize domestication of the farmed salmon to reduce the problem of genetic introgression with wild salmon. This can be done by reducing its ability for survival in the wild and interaction with wild salmon, using sterile fish (i.e. triploid fish or DNA vaccinated) or by breeding for traits affecting survival and mating success in the wild negatively.

Whether or not the escape of salmon strains with delayed or inhibited sexual maturation (developed using polyploidy, MAS, methods of genetic engineering, DNA vaccines or traditional breeding) will have any ecological effect or impact beyond what is seen from today's escapees needs to be scientifically substantiated through experimental approaches before approval is given for such fish in sea-based aquaculture.

It is likely that permanently altering the genome of the domestic salmon through focused breeding efforts or genetic engineering (i.e. heritable changes) in order to delay maturation will have a greater impact on wild populations through hybridization, than affecting maturation through controlling external parameters such as light, temperature and feed or polyploidy. Hence, for the farms choosing to use salmon with maturation delayed or inhibited by either MAS or genetic engineering, there is a need for further precautionary measures and regulation compared to those that farm salmon using external parameters (environmental cues). This implies that within sea-based aquaculture there is a need for scientific studies looking at how potential future salmon strains with delayed or inhibited maturation might impact wild strains, and if the introduced changes affect behavior, physiological or metabolic characteristics and hence adaption to the environment. For example polyploidy inhibits gonadal production in female salmon, i.e. they will not sexually mature and are unlikely to enter rivers. Male polyploids will develop gonads, but the sperm produced will not be viable. Male triploids are thus likely to swim up-river and partake in mate competition and mating's, however, any eggs fertilized by these males will be non-viable. As mentioned previously triploid males might be larger than diploid males and thus seen as a preferred mate by female salmon. The implication is that escaped triploid salmon could have substantial adverse consequences on vulnerable salmon populations.

Depending on the method used and its specific impact on sexual maturation, the experience with farmed fish and their impacts on wild salmon today can be used to some degree to predict potential levels of interaction and impending effects. This experience can be used for comparing if they either increase or decrease the environmental impacts with what is reported from today's domesticated salmon. Though it might seem quite severe when such a significant trait is disturbed or altered, the core issues related to environmental impacts are very much the same as for today's domesticated salmon: any substances provided through the feed might end up in the environment and the farmed salmon will escape unless kept in secure land-locked facilities.

4.3 Three hypotheses about ecological impacts by GM salmon escapes

Three specific hypotheses have been developed describing potential ecological effects from escaped GM fish (Muir and Howard, 1999, Muir and Howard, 2001, Muir and Howard, 2002):

The Trojan gene: if the transgenic fish has enhanced mating success, but reduced genetic viability compared to the wild type, the transgene(s) will quickly enter into the wild populations. Because individuals with reduced fitness (efficiency to cope with pertaining and changing conditions) may obtain the majority of the mating, the hybrid population will subsequently experience a decline leading to a potential loss of wild stocks.

The Purge: if the transgenic fish has lower fitness than the wild type, hybridization events will cause the transgene(s) to be purged from the species through natural selection and the wild stocks may persist.

The Spread: if the fitness of the transgenic fish is equal to or exceeds that of its wild type, conspecifics hybridization will cause the transgene(s) to enter and establish in the wild population, together with other genes from the domesticated fish. Since the domesticated populations vary little in their genetic make-up, and will invade several rivers, this could lead to a loss of genetic diversity in the wild salmon (Glover et al., 2012, Glover et al., 2013). Over time, this can increase susceptibility to disease and compromise the genetic adaptation to specific natal waters.

The three hypotheses listed above could be applied not only to any GM salmon, but also to salmon strains where other methods for genetic alteration, DNA vaccines, MAS or traditional breeding have been used.

The influence of genetically altered salmon on wild populations will depend on its modified traits and the resulting physiological, metabolic and behavioral changes. It is difficult to predict how a late- or non-maturing GM salmon variety for aquaculture would affect wild populations. The impact may depend on whether sexual maturation is delayed, or partially or completely inhibited. Maturing late could mean a loss of fitness as the late maturing salmon would spend more time at sea, where mortality is high, before ascending a river. However, if it survives until maturation, the late maturing salmon could be larger than wild conspecifics and thus have a competitive advantage at the mating ground because larger females produce more eggs, and larger males gain more mates. A partially maturing salmon could be drawn up-river with the potential of participating in mating's, producing non-viable offspring. In both these cases, the modified salmon would also compete for resources such as habitat and prey with according adverse consequences for the wild salmon strain and other organisms inhabiting the river. A non-maturing salmon is unlikely to run up-river (however, this will depend which specific physiological processes of maturation that are inhibited), and thus will have little interaction with wild salmon and river ecosystems. As such, it appears that complete inhibition of maturation is superior to delaying maturation, though the method used and the resulting fish should be thoroughly risk assessed.

For now, the production of the *AquAdvantage* salmon is limited to land-locked facilities (in Panama and Canada), including both physical and biological confinement barriers, in areas were survival would be difficult, if not impossible, for the salmon in case of escape (Van Eenennaam and Muir, 2011). Approaches that combine GM traits with induced sterility or other approaches for inhibiting reproduction (as in the case of the triploid *AquAdvantage* salmon) could be used to minimize the risk of genetic impact after escape. Still, the impact of other types of interaction with wild salmon and the environment should not be underestimated.

MAS shows promise in the development of disease resistance, which could result in a spread-scenario. It can be argued that containment measures should equally be applied to any domestic salmon strain where there is suspicion of a reproductive or survival advantage in the wild.

To conclude this section emphasis must be placed on the difficulty of evaluating environmental effects in general for salmon where the process of sexual maturation has been inhibited or disturbed. Although triploidy provides a relatively clear case, the various approaches to genetic alteration of the process of sexual maturation and the large number of potential targets for selection or modification makes it difficult to provide anything more than generalizations of potential effects. Since the genetic diversity of wild Atlantic salmon is at present under high pressure from escaped farm salmon (Glover et al., 2012, Gausen and Moen, 1991), the introduction of salmon with delayed maturation must be accompanied with precautionary measures to avoid escape and other impacts on the environment until more scientific understanding of potential adverse environmental effects is gained.

5 Ethical questions

Evaluating the ethical issues at stake when utilizing new technology is not merely a practice of calculating the risk of adverse effects and weighing these against the possible benefits, but also asking the question of what kind of technological development that will contribute to a better society (Myskja and Myhr, 2012) and whether the consequences will be local or global.

When evaluating ethical issues there are issues that need to be considered first:

i. Which considerations are ethically relevant? And for whom are they relevant?
ii. What should be the conditions for ethically acceptable use of biotechnology, e.g. when using biotechnology to change aquatic organisms?
iii. Who has the responsibility for making the decision?
iv. How to weigh the various implications and consequences for the involved parties against each other?

For example, important consideration may be that animals should have a decent life and that consumers have the right to good quality fish. These considerations may conflict with each other, and therefore animal welfare must be weighed against consumer interests. Very often these kinds of conflicts end in some form of compromise, occasionally one or more of the parties will be favoured and gain more from the compromise than other parties. Depending on the ethical framework and the approach considered, answers and the potential compromises to be made will differ. Utilitarianism could for example expected to be more supportive of MAS than genetic engineering due to the difference in number of suffering and sacrificed salmon for each approach. In addition, delayed or inhibited sexual maturation would improve upon the physical welfare on salmon in captivity. Taking a deontological approach would condemn any of these approaches as all involve treating the salmon only as means to more effective food production and question if the approach breaches with species integrity. An anthropocentric view would allow most approaches given that they do not pose a threat to human interest. In other words, what ethical directions that will guide the way forward will most likely be a compromise between various interests and different frameworks of ethics adhered to by different fractions of the industry, government, researchers, environmentalists, fish farmers and the public.

To assist in focusing on the relevant considerations and weighing implications and consequences for the involved parties, ethical matrixes can be built. Several examples focusing on biotechnology and aquaculture have been published in recent years (Kaiser, 2005, Kaiser and Forsberg, 2001, Kaiser et al., 2007, Mepham, 2006, Mepham, 2000, Bremer et al., 2015). Within such a matrix the different interest groups, parties or entities that may be impacted by the issue at hand are listed on one axis and ethical principles such as “beneficence”, “non-maleficence”, “autonomy” and “justice” on the other axis. Application of a top-down approach entails that the facilitators set the matrix, while a bottom-up approach allows the participants to modify the matrix in accordance with their own perception of the issue at hand. Bremer et al. (2015) facilitated workshops in Norway, Germany and the United Kingdom asking stakeholders to discuss and deliberate on ethical issues raised by GM Atlantic salmon (*AquAdvantage*) using the ethical matrix tool. The study had a bottom-up approach, which led to the inclusion of the scientific community as one of the stakeholders as the participants felt that the scientific community did not necessarily belong with the industry. The different roles and interests of stakeholders were also emphasized, for example the role of an individual both as a consumer of marketed goods, and as a citizen participating in the shaping of society and politics. It is important to recognize that different roles and interest can often be conflicting, even for individual stakeholders. Although each workshop developed unique perspectives, the workshop groups as a whole recognised the uncertainties connected to GM salmon and favoured a precautionary approach (Bremer et al., 2015). Additionally, the participants weighed the welfare of salmon against the potential benefits for other stakeholders (industry vs. GM developers, big companies vs. small companies, consumers vs. environment), and found autonomy to be of importance to all stakeholders. The interest of the present versus the future was also discussed.

Table 2 gives an overview of benefits, ecological issues, and ethical aspects linked to the different approaches described for delaying maturation in salmon.

5.1 Solving challenges for the aquaculture industry

The various approaches described above are applied, or can be applied by the aquaculture industry and farmers in the future. Many academic institutions and research institutes are currently researching how to effectively delay maturation in salmon. Most of the approaches, with the exception of environmental and physiological manipulation, are employing methods that are of relevance also for reducing disease, reducing impacts on wild salmon and for increasing growth. In addition to environmental and physiological manipulation the use of polyploidy and MAS is increasing within aquaculture. Other approaches such as genetic engineering and DNA vaccines may be accessible to the industry in the future, but is dependent on approval through regulatory processes. The approach and time usage of the regulatory process and its outcome are of high relevance for the industry and the farmers in order to reduce economical risk and to ensure stability of production and income.

In addition to national regulations concerning research and fish farming, there are as in other industries a growing interest within the aquaculture industry to implement corporate social responsibility and sustainability. One international initiative is for example the Aquaculture Stewardship Council (ASC) that has developed standards for responsible fish farming including salmon (Aquaculture Stewardship Council, 2012). Although the industry is not compelled at present to adhere to such standards, the first companies have now been certified according to the ASC standards. This includes criteria for environmental and social responsibility, and a requirement for transparency. Implementation of such standards are important for building trust among consumers and it is important that they are acknowledged when introducing new approaches that may affect the animal welfare, the environment or sustainability. The benefits and relevance of the different approaches to delaying maturation in salmon for consumers, researchers and developers, industry and the farmers are briefly described in Table 2.

5.2 Fish welfare – an ethical aspect of breeding

The word ‘welfare’ is derived from well + fare, i.e., how well (or dignified) an animal ’fares‘(travels) through life. According to Broom (1996) a definition of animal welfare can be: “The welfare of an animal is its state as regards its attempts to cope with its environment”, i.e. mainly focused on the metabolic and physiological state of the animal and its ability to maintain homeostasis. There are other definitions of welfare with other focuses such as the animal’s subjective experience of its condition (feeling based) and/or on whether it as an individual or as a population can lead a natural life and maintain itself within its natural habitat and its changes (Bergqvist and Gunnarsson, 2013, Stien et al., 2013, Ashley, 2007, Lund et al., 2007). Animal welfare includes more than the absence of suffering as it also encompasses positive welfare (Boissy et al., 2007), implying that denying an animal all positive experiences and stimuli is also an ethical problem. It has been reported that most fish possibly possess a degree of sentience (Brown, 2015, Chandroo et al., 2004), and the notion that fish are sentient should not be ignored when weighing the different arguments of the ethical matrix.

When breeding for specific traits it is important to ensure that the selection of these traits do not coopt for traits affecting animal health and welfare negatively. The broader the breeding goal is, the more difficult it is to succeed, and there is a higher risk of inbreeding and genetic erosion. This is the case for many breeding programs selecting on easily measurable traits (i.e. external traits) of individual fish. Advanced family based selection programs will often perform better in the long run, because they consider more information. By implementing MAS in the breeding program the sought after genotype could be selected, rather than the phenotype. This is likely to make selection for recessive alleles easier. Genetic engineering would skip the need to select for the wanted trait, but would require generations of breeding in order to eliminate potential negative effects and for ensuring the stability of the genetic construct. Independently of the approach chosen, it is always important to consider the trade-offs between production efficiency for the fish farmer and the animal welfare when selecting for specific traits. It should be noted that the prioritization of productivity as for example growth over animal welfare is a general ethical issue within the food production industry, and not a result of new technology pushing the borders of what can be achieved (Christiansen and Sandøe, 2000).

Independent of the approach chosen for breeding for delayed maturation, it must be in accordance with promoting fish welfare. As salmon mature stress is increased, they lose weight and become more aggressive towards their conspecifics (Taranger et al., 2010, Ashley, 2007, Bergqvist and Gunnarsson, 2013). Open sores and damage to fins and forks due to aggressive interactions are detrimental to fish health and welfare (Bergqvist and Gunnarsson, 2013, Ashley, 2007). Maturation in seawater is also problematic because the ability to hyper-osmoregulate is lost, which in turn leads to dehydration and death, unless the fish is moved to fresh water (Stien et al., 2013, Taranger et al., 2010). While wild salmon migrate up their native rivers, domestic salmon is usually kept in sea pens until slaughtered, regardless of maturation state. Removing a fish from water induces a high level of stress and a physiological emergency response in the fish (Ashley, 2007). Methods for delaying or inhibiting maturation that would require more handling could increase stress as compared to today's situation. However, if such procedures were combined with already established handling procedures (such as in connection to vaccination) it might be within an already accepted norm. Methods for delaying maturation that do not require more handling do not necessarily induce more stress, but other effects that could affect fish welfare should be investigated for frequency and effect.

5.2.1. Induction of triploidy and implications for welfare

Induction of triploidy is recognized as the most effective way of producing large amounts of sterile fish, and has been applied to many cultured fish species (Taranger et al., 2010). Induced triploidy is connected to health issues and higher sensitivity to sub-optimal rearing conditions (Taranger et al., 2010, Fraser et al., 2012a, Benfey, 2001, Fraser et al., 2013). Changes in brain morphology have also been documented (Fraser et al., 2012b). It has been indicated that several of these issues can be avoided by optimizing the triploids rearing conditions and diets. Malformation in triploid salmon have serious ethical implications if fish are considered to be sentient creatures, and not only self-replicating machines. If one is able to rear triploid fish without compromising their physical health this can be a good strategy for avoiding stress and health issues related to maturation. More research into the salmon's cognitive abilities is, however, necessary to properly evaluate the potential impact of the changes in brain morphology (Fraser et al., 2012b).

If the induction of triploidy is viewed as unethical, the conscious consumer might decide not to purchase products derived from triploid salmon. Replacing diploid fish with triploid fish might yield economic losses for the producers if measures are not taken to optimize their rearing conditions and welfare (Fraser et al., 2013). Such measures might influence consumer's choice.

5.2.2. Welfare issues connected to GM salmon

Genetically modifying salmonids in order to reach a breeding goal could have several unforeseen health and behavioral consequences for the fish (Devlin et al., 2006, Devlin et al., 2014, Panserat et al., 2014, Leggatt et al., 2012, Devlin et al., 2009a, Devlin et al., 2009b, Sundt-Hansen et al., 2007, Mori et al., 2007, Leggatt et al., 2007, Vandersteen Tymchuk et al., 2005, Devlin et al., 2004, Bessey et al., 2004) through pleiotropic effects. Detrimental health effects such as skeletal deformations, compromised immune systems and altered organ structures are not compatible with good animal husbandry. If the goal of genetically modifying fish is to quickly fulfill breeding goals as for example increased growth, the result might be production of many unhealthy animals. However, genetic engineering technology is advancing quickly. Increased understanding of processes and improved specificity could eventually produce fish with less off-target effects (Edvardsen et al., 2014). It is important when genetically modifying one trait, such as timing of maturation, that the overall performance and welfare of the fish is evaluated in case of unforeseen and negative effects.

Finally, in the Norwegian debate on the issue of delaying or preventing maturation in Atlantic salmon, the Norwegian Act on Animal Welfare must be considered. The Norwegian Act on Animal welfare (2009) states that one should not breed against an animal's ability to practice natural behavior. In this regard it can be discussed if it would be more ethically acceptable to allow fish to mature under aquaculture conditions with the negative consequences this has for welfare (i.e. allowing natural behavior), or if it would be acceptable to breed the salmon in order to adapt it to the farm environment, including attempts to delay maturation as this would benefit the general welfare.

MAS, being the technology most closely resembling traditional selection, might be the technology where the least adverse effects are expected to arise as changes are not immediate and drastic compared to polyploidy and genetic engineering. Also, this technology relies on the selection of genes in their natural context, likely to provide the appropriate scaffolding. However, pushing certain traits too far in one direction or the other could still have serious welfare implications (e.g. the Belgian Blue Cattle, fast-growing chickens, and certain brachycephalic dog breeds), and the success and acceptance of MAS derived salmon strains will rely on the responsibility and effort of developers to produce healthy salmon.

5.2.3. Intrinsic value and species integrity

Research on animals entail two important ethical questions: (i) what, if any, are the moral considerations restricting or encompassing such research, and (ii) is it morally legitimate to use animals merely as a resource or means to meet human needs? The question concerning animals' intrinsic value has been especially challenging. The recognition that animals have an intrinsic value calls for carefully considered decisions and actions, and further implies that any interactions that may cause harm to the animal becomes a moral issue. The Norwegian Act on Animal Welfare(2009), states that animals have an intrinsic value. This inclusion clearly demands that animal welfare must be prioritised irrespective of the value the animal may have for humans.

Other important ethical questions might relate to respect for species integrity i.e. do we as humans have the right to transform this species to better suit our needs?, or what is the acceptable level of risk to impose on the wild salmon populations and their surrounding ecosystems in order to achieve benefits for humans (Ormandy et al., 2011, Vries, 2006). While the former mainly relates to breeding and modification of animals, the latter question is also highly relevant for issues relating to pollution and damming of rivers, among others. It will be difficult to determine what is to be considered as serious interference with a species, and what should count as serious harm. For example, the Norwegian Act on Animal Welfare (2009) demands that animal breeding shall encourage characteristics that give robust animals and that have good welfare. Reproduction, including genetic engineering, shall not be carried out in such a way that it:

- changes genes in such a way that they impair the animals' physical or mental functions in a negative way, or continues pursuing such genes
- reduces the animals' ability to practice natural behaviour
- stimulates general ethical reactions

The formulations here are clearly intended to conserve and protect both the integrity of the species and wellbeing of individual animals.

5.3 Ethical issues related to patenting and licensing of salmon genes

There has been a strong policy to encourage patenting in research and development projects by governments and research funding institutions (Grimm and Jaenicke, 2012), an activity that is also stimulated by genetic engineering applied within pharmacology and medicine as well as with GMOs. Hence, it can be argued that genetic engineering is a major driver towards more use of IPR and in particular patenting (Rosendal et al., 2006, Rosendal, 2006). There is an ongoing debate on the ethical issues related to patenting living organisms and their genes. Major questions revolve around the autonomy and integrity of animal species, and whether or not it is acceptable to use and transform animals. Another issue pertains to the exclusiveness and protection offered to patented organism and whether or not it is right to keep knowledge related to living organism under patent. This debate is highly relevant for aquaculture, since the increased knowledge about the genome of economically important fish species and biotechnology opens for a large array of potential applications. Drawing upon experiences from the agricultural sector patent protection has become the norm for GM plants (i.e. subject to insertion of new or modified gene varieties), while plants modified through traditional methods, despite also utilizing advanced tools and new knowledge, are not offered the same protection. It might be easier for the industry to guard GM strains of salmon, than strains developed through breeding, because they can easily be distinguished from all other strains by the insertion of the genetic construct. However, when it comes to smaller modifications of endogenous genes through targeted mutagenesis, enforcing patents could prove to be challenging because it would be extraordinarily difficult to distinguish between engineered changes and random genetic changes. One suggestion for enforcing patent and other ownership rights is DNA fingerprinting and certification of breed and production stocks, so that uncertified production could be discovered and documented (Olesen et al., 2008).

A separate, but similar issue is the patenting of MAS procedures, including the genetic markers. Access to this technology could be limited only to those developers and breeders that can afford to pay the fees connected to the use of specific markers or selection processes, effectively skewing competition in favour of the larger companies and potentially reducing the diversity of domestic salmon strains. However, MAS markers have a limited life span, as they are only useful until the targeted gene has become integral to the population (Olesen et al., 2008).

Previous research into the Norwegian salmon sector has shown that emerging sets of legislation had low visibility among the relevant actors, but also that most of them were becoming increasingly concerned with and interested in questions of access and exclusive rights to improved breeding material from Atlantic salmon (Olesen et al., 2007). Most Norwegian breeders are confident in the superiority of their own salmon strains, and the good international reputation of Norwegian salmon. However, the genetic material of the Norwegian Atlantic salmon (both wild and domestic strains) have been spread to a great number of breeders worldwide, and worry has been raised that the Norwegian aquaculture industry in the future might become dependent on foreign companies for access to new and improved genetic material of Atlantic salmon (Olesen et al., 2008). The breeders also acknowledge their vulnerability, in the case of new and improved materials or traits becoming severely restricted due to IPR and the application of royalties. The predominant view among actors in Norwegian aquaculture is that the sector needs to find a balance between access to breeding material and protection of own innovations (Olesen et al., 2007). Current patent legislation hence balances on a thin line between being an incentive for development and innovation and restricting the very same through monopolization and prevention of access.

Another debatable issue arising from patenting is the patenting of publicly funded research findings by commercial companies raising the important ethical question concerning access to publicly funded research.

If a salmon with delayed maturation, or a technique for delaying maturation through feed additives or vaccines is developed, it is important that there exists a legal trade framework that includes the interest of developers, breeders, farmers and consumers. Within the existing laws a new type of feed, a MAS method, a DNA vaccine or GM modification can be patented, however, a salmon developed through normal breeding or MAS cannot. Thus, for developers it might be beneficial to choose GM rather than traditional or MAS breeding when initiating the improvement of a specific trait. It is clear that the legal framework surrounding the development and introduction of traits and salmon strains could affect the approach chosen by the developers. In the current climate, genetic modification or feed additives and vaccines are the developers' best options with regards to ownership and rights.

5.4 Societal and consumer considerations

New strategies and methods for breeding may increase production efficiency and perceived physical and biochemical quality of farmed fish. At the same time these novel methods might lead to consumer scepticism and market resistance due to for example ethical concerns, the use of genetic engineering or the use of chemical compounds such as hormones. Together with regulatory constraint this might cause difficulties for the aquaculture industry. Although these novel methods might produce similar products, consumer acceptance will probably rely very much upon the method used to achieve the delay of maturation in salmon. It can be proposed that manipulation of the external environment in the form of adjusted photoperiod (which is used extensively already to produce off-season smolt) is more acceptable to consumers than GM salmon due to the perception of what is "natural" and what is artificial. See table 2 for an overview of relevant societal and consumer consideration for the different approaches described for delaying maturation.

Salmon bred in a traditional way where genetic changes are induced over generations of selection for later maturation would probably also be much more acceptable, as long as the salmon appears healthy and no severe side-effects are observed (even though naturally breed, the Belgian Blue cattle bred has little consumer acceptance in several countries due to their enormous muscle mass with correlated health problems for the animals). Since fish in general are viewed as less sentient than mammals, and the phylogenetic distance between the fish and human causes less emotional attachment towards fish than towards animals, consumers may protest less against breeding and culturing methods that affect the welfare of salmon (Kupsala et al., 2013). Salmon given hormonal supplements or vaccines and medication in order to delay maturation would probably lead to concerns regarding effects on the consumer and the environment (i.e. the current debate on use of antibiotics in farming industries).

Surveys performed in Scandinavia in order to study preferences show that Scandinavian citizens in general are highly skeptical to GM food, and are willing to pay an additional cost in order to purchase non-GM food (Hursti et al., 2002, Chern et al., 2002). In a particular survey performed by Chern et al. (2002) consumers were willing to pay almost 70% more for non-GM salmon compared to GM-salmon. Younger people seem to be more positive to GM-products in general, and thus, the majority opinion regarding GMOs might change with time (Hursti et al., 2002, Magnus et al., 2009). Despite the Norwegian population being among the most positive to modern biotechnology in Europe, it is clear that the positive attitude is mainly directed at biotechnology used for medical purposes, and not so much at GM technology in food production (Hviid Nielsen, 2012). On the other hand, consumers may consider modification of traits that reduce production cost (lower feed requirements, shorter production time etc.) favorably if this will make salmon cheaper and more accessible (Smith et al., 2010).

Informative product labeling and freedom of choice seem to be important for most consumers (Chern et al., 2002, Grimsrud et al., 2013). Salmon products from GM salmon will need to be labeled in countries such as Norway that have implemented GM regulations. The same applies if GM salmon is imported from countries without GM legislation to countries with GM legislation. Although there is no specific regulation for labeling GM products in the US, the FDA has decided to hold up the approval of *AquAdvantage* salmon until such a system is in place. This decision by the FDA may affect consumer acceptance of the *AquAdvantage* in the USA, but will be according to consumer rights to freedom of choice. How new developments within genetic engineering such as CRISPR/Cas and DNA vaccines will be regulated, and if the final product will be labeled as a GMO, is uncertain. It is therefore of high importance that the aquaculture industry, the farmers and the researchers strive to bring forward information and uphold transparency with regard to approaches under development, so that the ecological and ethical issues can be brought forward and discussed publicly. This is especially relevant for the developing technologies. Such transparency can also increase the robustness of the approaches by early consideration of issues presented by consumers and citizens. Though several NGO’s speak for consumers and citizens, they have less influence and power than the institutions and companies representing research, development and industry, and have particular little influence when it comes to ethical concerns. In Norway, a consumer council has been established, and in a few other countries there are non-governmental consumer bodies. However, their mandate does not include bringing forward consumers concerns into decision-making. Hence, how to acknowledge consumers concerns in an institutionalized way needs to be addresses at a national level, and ultimately at a supranational level.

6 Summary and conclusion

There are several technologies and methods available to the salmon industry for delaying and possibly preventing maturation of farmed salmon (table 1), some are in use, while others require further research effort and refinement. The way forward will to a high degree depend on the aquaculture industry, the channeling of public research funds, governmental incentives and regulation, and finally consumer acceptance.

Currently there exist several knowledge gaps connected to the herein discussed approaches. For some of the technologies such as MAS, DNA vaccines and GM, there are still questions about feasibility and level of effectiveness in altering the maturation process. Whereas for other approaches, such as photo-manipulation and induction of triploidy, the main issues are related to animal husbandry laws and consumer acceptance. GM technology also raises several issues when it comes to consumer acceptance, regulations and risk assessment. When discussing breeding, using traditional methods or MAS, triploidy and GM of salmon, another important and critical issue relevant to all approaches is the potential threat that these late maturing or non-maturing fish could pose to the environment and to the wild strains of Atlantic salmon. This ecological threat is critical since wild salmon already is under pressure from several sources (pollution, climate change, fisheries, habitat loss etc.), in addition to impacts from aquaculture. Due to the difficulty of evaluating environmental effects in general for salmon where the process of sexual maturation has been inhibited or disturbed, the precautionary principle must be employed to avoid escape and other impacts on the environment until more scientific understanding of how to reduce potential adverse environmental effects is gained.

Ethically, the domestication process and intensive farming is already interfering with the autonomy and welfare of farmed salmon. Salmon is currently being used as a means to an end, namely food production, and the main question is thus how a delay in maturation could worsen or improve upon the current situation of domestic salmon. It is likely that arguments based on animal welfare, species integrity, environmental health, the use of the patent system, and consumer acceptance will be put forward by NGOs and other organizations representing consumers and the environment in order to affect the industry’s choice of approach and the outcomes of governmental regulation processes.

Consumer opinion is important. In general there is high skepticism towards GM-food, and increasing consciousness around food and feed additives (for examples conservatives and antibiotics), particularly in the Scandinavian countries. Consumers are also becoming more aware of and interested in the animal welfare aspects of food production. Consumer opinion, acceptance and rights are major factors within the food sector, and it will be important for the aquaculture industry to satisfy the majority of consumers. The aquaculture industry, the farmers and the researchers must therefore bring forward information and uphold transparency with regard to approaches under development, so that the ecological and ethical issues can be brought forward and discussed. This transparency may increase the robustness of the approaches by bringing forward different perspectives that will give a broader basis for making decisions by both the industry and policymakers. Consumer interest should also be protected through easy access to information about development, origin, and production methods, i.e. the implementation of a labeling system.

There is a clear need to establish a legal framework for the regulation of IPR and patenting that takes into consideration the potential of modern biotechnology. Preferably, this should be developed before problems and conflict have the possibility to arise, as developing regulations under such circumstances would be complex, because some precedence might already be established for certain aspects of IPR regulation. The regulations should as far as possible try to acknowledge the interests of the breed stock developers and the farmers, so that neither part is left in an inferior position. The ethical issues related to patenting living organisms and their genes needs to be extended to include patents on biotechnology approaches used on salmon.

It is also important that NGOs and the general public, that are not directly involved in the aquaculture industry, are heard in relation to regulations governing the specific sector, as such could have far-reaching consequences for future regulation of other similar sectors such as animal husbandry.

Will industry, government or consumers ultimately decide the way forward together? Different approaches and solutions might be of variable suitability to different markets. It is therefore important that both the benefits and the potential ecological and ethical issues are identified and discussed in open and participative ways. An institutionalized dialogue addressing the interest of all parties would provide a good basis for making decisions on the future of aquaculture and the use of biotechnology within the industry.

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Table 1 An overview of different biotechnology based methods and tools that can be used to delay or prevent maturation in farmed Atlantic salmon.

Technology	Objective	How	Purpose
Chromosome manipulation (polyploidy, monosexing: gynogenesis, androgenesis)	Manipulating the origin and number of chromosome sets of the salmon	By mating tetraploid salmon with diploid salmon, or shocking fertilized roe at a certain developmental stage	Making polyploid fish that are sterile, and have continued growth.
Marker-assisted selection/breeding	Markers associated with certain preferential or unwanted traits (genes) are selected for or against	By markers that are either morphological, biochemical, cytological, DNA-based, or otherwise molecularly based	Makes it easier to select for traits that 1. are difficult/expensive to quantify, 2. only develop late in life, 3. have low heritability
Genetic modification, or targeted mutagenesis	The transferring of a gene from one species to another in order to induce a new trait, or by changing gene activity in salmon	By genetic engineering technologies and targeted mutagenesis protocols	Can be used to introduce genes across species borders. Controlled targeted mutagenesis can immediately introduce a trait in the next generation without breeding.
DNA vaccination	DNA-based vaccine designed to disrupt sexual maturation processes	By targeting relevant genes/proteins and disrupting their function to partially or completely inhibit sexual maturation	Make DNA vaccines that can expand already established vaccination program.
-Omic technologies; studying genes, proteins and the metabolic pathways	Tool for characterization of brood stock, tracking of families, achieving increased understanding of fish physiology and immunology, quality trait analysis and selection, as well as for authentication	Genetic linkage maps Radiation hybrid maps Quantitative trait loci (QTL) MAS, genome wide association studies (GWAS) Transcriptomics Proteomics Protein arrays Metabolomics	Can be used to gain information that can be applied for improving selection processes, feed composition and fish health

Table 2 Benefits as well as ecological and ethical aspects in relation to technologies and methods for preventing early maturation in farmed salmon

Approach	Benefits	Environmental risks	Societal and consumer issues	Current status
Traditional breeding	▪ Acceptable to consumers	▪ Breeding with wild populations	▪ Possible negative effects linked to breeding for a specific trait	▪ Long history of use ▪ Well-established programs in Norway
Environment manipulation (Light and diet)	▪ No adverse physiological effects from light regimes ▪ Acceptable to consumers	▪ Escaped fish can breed with wild populations	▪ Ethical and animal welfare questions related to different diet regimes	▪ Photo-manipulation used in early life stages to stimulate growth of off-season smolt
Physiological manipulation	▪ Higher efficiency ▪ Can be combined with feed or vaccination efforts	▪ Vaccines and hormone additives affect other marine creatures ▪ Escaped fish can breed with wild populations if maturation is not completely blocked	▪ Requires food additives, or more handling of the fish if substances require injection ▪ Hormone additives might not be acceptable with consumers	▪ In early stages of research
Biotechnology **I.** *Induced polyploidy*	**I.** ▪ Fish are sterile ▪ Fish expected to grow faster due to no investment in maturation	**I.** ▪ Almost 100% effective, but some escapees may still be able to reproduce. ▪ Interaction with wild fish	**I.** ▪ Consumer skepticisms ▪ Many ethical and fish welfare considerations	**I.** ▪ Established methods ▪ Widely used in other marine species ▪ Currently tested in Norwegian aquaculture

II. *Marker assisted selection*	**II.** ▪ Targets genotype rather than phenotype ▪ Allows for more precise selection of breeding stock	**II.** ▪ Effects of interaction with wild populations are difficult to predict ▪ Escaped fish can breed with wild populations ▪ Potential for driving wild populations towards late maturation due to hybridization	**II.** ▪ Requires specialists on a large scale within the breeding industry	**II.** ▪ Promising ▪ Salmon genome recently mapped ▪ Currently used for IPN resistance mapping and MAS
III. *Genetic modification*	**III.** ▪ Highly specific with regards to gene selection	**III.** ▪ Escaped fish can breed with wild populations ▪ Uncertainty connected to spread of novel genes	**III.** ▪ High consumer skepticisms ▪ Strict requirements in regulation ▪ Many ethical and welfare related issues	**III.** ▪ Experimental ▪ Approved aquarium fish ▪ *AquAdvantage* under approval
IV. *DNA vaccination*	**IV.** ▪ Can be implemented in already established vaccination programs	**IV.** ▪ Unknown mobility of DNA plasmids in the environment ▪ Uncertainty related to what the targets for vaccination should be	**IV.** ▪ Consumer skepticisms ▪ Requires studies to meet requirements in regulation	**IV.** ▪ Potential vaccine targets have been identified ▪ Will require extensive experimental testing before approval by authorities